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Sunsafe bryophytes: photoprotection from excess and damaging solar radiation

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Abstract
Whilst light is essential for photosynthesis and development of plants, both excess photosynthetically active radiation and certain wavelengths (e.g. high energy ultraviolet-B) radiation can be damaging. Plants in general possess a suite of mechanisms that act to either prevent absorption of damaging and excess radiation or to mitigate against the damage that such radiation can cause once it is absorbed. Whilst bryophytes share many of these photoprotective mechanisms with the vascular plants, there are key differences in the photoprotection available to bryophytes. Some of these differences pertain to structural features, such as protective epidermal layers, that are available to vascular plants but not generally to bryophytes. Bryophytes thus have to invest more in cellular level photoprotection than vascular plants. In other respects bryophytes may retain mechanisms found in algal ancestors (e.g. thermal energy dissipation associated with the LHCSR protein) that have been lost during the evolution of vascular plants. Many bryophytes are able to manage light absorption during desiccation and rehydration and freezing and thawing, resulting in potentially novel mechanisms of energy dissipation. Given the high stress environments that many bryophytes inhabit, from hot or frozen deserts to alpine habitats with high incident UV-B radiation, it is unsurprising that they have a suite of photoprotective strategies.

Keywords
sunsafe, photoprotection, bryophytes, excess, damaging, solar, radiation

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Sunsafe bryophytes:
Photoprotection from excess and damaging solar radiation

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Summary

Whilst light is essential for photosynthesis and development of plants, both excess photosynthetically active radiation and certain wavelengths (e.g. high energy ultraviolet-B) radiation can be damaging. Plants in general possess a suite of mechanisms that act to either prevent absorption of damaging and excess radiation or to mitigate against the damage that such radiation can cause once it is absorbed. Whilst bryophytes share many of these photoprotective mechanisms with the vascular plants, there are key differences in the photoprotection available to bryophytes. Some of these differences pertain to structural features, such as protective epidermal layers, that are available to vascular plants but not

generally to bryophytes. Bryophytes thus have to invest more in cellular level photoprotection than vascular plants. In other respects bryophytes may retain mechanisms found in algal ancestors (e.g. thermal energy dissipation associated with the LHCSR protein) that have been lost during the evolution of vascular plants. Many bryophytes are able to manage light absorption during desiccation and rehydration and freezing and thawing, resulting in potentially novel mechanisms of energy dissipation. Given the high stress environments that many bryophytes inhabit, from hot or frozen deserts to alpine habitats with high incident UV-B radiation, it is unsurprising that they have a suite of photoprotective stategies.

Abbreviations:

PSII	photosystem II
UVAC	ultraviolet-B absorbing compound
UV-B	ultraviolet-B
L/Lx	lutein/lutein epoxide
NPQ	non-photochemical quenching
ROS	reactive oxygen species
PAR	photosynthetically active radiation

I Introduction

Light provides the energy source for photosynthesis and is essential for all plants, however, certain wavelengths, especially ultraviolet-B (UV-B) radiation can cause direct damage to the photosynthetic apparatus especially photosystem II (PSII). The challenge facing photosynthetic organisms is therefore to optimize light absorption for photosynthesis while avoiding damage. Plants have evolved a number of strategies to tailor light absorption to the capacity for utilization by photosynthesis and to either protect themselves from photodamage or repair any that occurs (Takahashi and Badger, 2011). Photoprotection occurs at a range of scales from processes at the molecular level such as dissipation of absorbed light energy as heat (Demmig-Adams and W. W. Adams, 1992; Niyogi, 1999; Nichol et al., 2012) to organ level mechanisms e.g. leaf movements and shading of radiation by waxes and hairs and screening pigments (Robberecht and Caldwell, 1978; Ehleringer and Cook, 1987; Robinson et al., 1993; Barker et al., 1997; Karabourniotis and Bornman, 1999).

The energy to drive photosynthesis comes mainly from the visible spectrum (400–700 nm). However, solar radiation also contains ultraviolet (UV) radiation, which is absorbed by plants

and can damage a range of biomolecules including DNA, RNA, proteins and PSII. Ultraviolet radiation increases naturally with altitude and decreases with latitude but has also been anthopogenically increased in polar regions, as a result of the ozone hole (McKenzie et al., 2007). Bryophytes are the dominant plant species in many of these high UV environments (alpine and polar regions; see Antarctic Chapter this volume) and appear to be generally well protected from the damaging effects of UV-B radiation (Newsham and Robinson, 2009).

Recent work suggests that primary photodamage to the photosynthetic apparatus occurs through direct absorption of light by the manganese cluster in the oxygen-evolving complex of PSII, with UV wavelengths followed by yellow wavelengths being most damaging (Takahashi et al., 2010). Primary photodamage to PSII is thus prevented by avoiding exposure to the damaging wavelengths, rather than dissipating the excess energy once it has been absorbed. Excess photosynthetically active radiation (PAR) absorbed by the light-harvesting complexes can still lead to production of reactive oxygen species (ROS) and so mechanisms that prevent ROS accumulation also play a role in photoprotection (reviewed in Takahashi and Badger, 2011). Whilst some photoprotective mechanisms offer cross protection by screening both visible and UV radiation, terrestrial plants also have a range of specific strategies to protect themselves from UV radiation.

As photosynthetic organisms, bryophytes therefore need to optimize light utilization but also protect their photosynthetic apparatus from damage. Although, many bryophytes can avoid damage by virtue of their environmental niche, for example those that grow in shady forests and other low light environments, some exist in open environments that combine high radiation with other potential stressors such as high temperatures and desiccation. Even shady environments can have a variable light regime, with sunflecks potentially supplying excess light to the chloroplasts (Watling et al., 1997). The absolute quantity of excess light depends on the photosynthetic capacity of the plant. Plants adapted to growth in high radiation environments will have high photosynthetic capacities, and thresholds for excess light will be greater than in plants adapted to low light, with correspondingly low photosynthetic capacities. In addition plants are usually able to cope with normal, diurnal fluctuations in light levels and can adapt to seasonal changes over time. Sudden increases present the greatest challenge to plants, for example the low- to high-light transition that occurs when a treefall gap is created in a rainforest (Lovelock et al., 1994). Often plants experience excess light because an additional environmental or biotic stress reduces their photosynthetic rate and therefore the

threshold for excess light is reduced. Whilst drought and temperature stress can impact photosynthetic rates in any plant species, many bryophytes have unusual physiological properties that could increase their risk of exposure to excess light. For example as water is lost from a desiccation tolerant moss the photosynthetic rate will decline (Chapters in this volume) and this will often coincide with exposure to high radiation, potentially increasing the requirement for photoprotection (Proctor and Smirnoff, 2011). Not surprisingly, tolerance to UV radiation exposure is often correlated with desiccation tolerance in bryophyte species (Csintalan et al., 1999). Phototolerance has also been shown to develop seasonally in desiccation-tolerant mosses, for example *Rhytidiadelphus squarrosus* shows greater tolerance to high light during dry summers than during the more humid winter and this tolerance can be simulated under laboratory conditions (Heber et al., 2006).

Plant protective strategies can be divided into those that operate to reduce light absorption and those that act within the leaf or photosynthetic organ to prevent absorbed light causing damage within the chloroplast.

II Avoiding absorption of excessive or damaging radiation

Bryophytes differ greatly from vascular plants in their morphology as they lack a protective cuticle and tissue differentiation (Gehrke, 1999) consequently leaving them more susceptible to photoinhibition and UV-induced damage (Fig. 1). Many external photoprotective mechanisms rely on structural features found in leaves of higher plants but not mosses, for example, external or epidermal screening through coatings or structures (e.g. wax and hairs; Ehleringer and Björkman, 1978; Robinson et al., 1993) or the ability of thick leaves to self shade lower cell layers (Robinson and Osmond, 1994). Avoidance type photoprotective mechanisms that could be employed by mosses include leaf orientation, self shading within the canopy, chloroplast movement and specific screening compounds.

A Generic screening mechanisms in bryophytes

Surface reflectance of moss turfs varies between species (Lovelock and Robinson, 2002) and also within species depending on the exposure to incident PAR and UV radiation (Robinson et al., 2005). Light attenuation through moss canopies varied six fold in *Pleurozium schreberi* collected from a range of habitats, showing that transmission characteristics are also plastic

(Rice et al., 2011). Reflectance from the moss canopy also increases as mosses desiccate reducing the quantity of light that can be absorbed and therefore lowering the potential for photodamage (Van Gaalen et al., 2007). Curling of stems of the desiccation tolerant pteridophyte *Selaginella lepidophylla* has been shown to reduce photoinhibition (Lebkuecher and Eickmeier, 1991) and this mechanism could also operate in mosses where drying and curling of leaves allows light to penetrate deeper into the canopy as less is intercepted by the top layer (Davey and Ellis-Evans, 1996; Zotz and Kahler, 2007; Rice et al., 2011). Chloroplasts can move within the cell to optimize light interception, as has been shown in the moss *Physcomitrella patens* (reviewed in Wada et al., 2003; Suetsugu and Wada, 2007, see chapter in this volume).

Compounds which act to screen specific wavelengths particularly UV-B radiation can be located within the photosynthetic cell itself or in the exposed epidermal layers. Within the typical leaf of vascular plants these sunscreens are often located in the epidermal layers but since most bryophytes lack such differentiation they will mainly occur within the photosynthetic cell (Lovelock and Robinson, 2002; Newsham et al., 2002; Newsham et al., 2005; Dunn and Robinson, 2006; Newsham, 2011). In some plants (Semerdjieva et al., 2003) and certain moss species they also accumulate in the cell walls (Fig. 2; Semerdjieva et al., 2003; Clarke and Robinson, 2008). Since most experiments concerned with the accumulation of UV absorbing compounds (UVAC) focus on methanol soluble compounds, accumulation of such compounds in the cell walls maybe seriously underreported. Since UVAC should also reduce damage to PSII their accumulation, location and effectiveness in screening the photosynthetic apparatus is an important aspect of photoprotection (Takahashi and Badger, 2011).

B Production of specific UV absorbing compounds in bryophytes

Both vascular and non-vascular plants produce secondary metabolites that can specifically screen out damaging ultraviolet radiation. A range of compounds with UV-absorbing properties including flavonoids, mycosporine-like amino acids, carotenoids, simple phenolics and hydroxycinnamic esters have been extracted and isolated from various organisms including several vascular plants, mosses, liverworts, phytoplankton, algae and cyanobacteria. Not only can these photoprotective compounds absorb UV light reducing the levels of harmful solar radiation reaching the photosynthetic apparatus and UV sensitive molecules

(Fig. 3) some, such as carotenoids and flavonoids, can also scavenge reactive oxygen species generated by UV radiation (Cash et al., 2007) preventing further UV-induced damage to DNA, proteins, membranes and PSII (section 4b). The composition of UVAC differs between organisms (Cooper-Driver and Bhattacharya, 1998; Rozema et al., 2002; Bjorn, 2007). Whilst flavonoids are the most common UVAC found in plants, and are ubiquitous in vascular plants, less than half of the bryophytes studied contain flavonoids (Markham, 1990; Cooper-Driver and Bhattacharya, 1998).

Comparison of studies into the impact of UV-B radiation on plants in general are often compounded by the methodology used; e.g. location (controlled laboratory conditions or field experiments) and sources of radiation whether natural fluctuating UV, solar radiation filtered through various screens or artificially produced using lamps that enhanced UV-B radiation levels (e.g. Caldwell and Flint, 1997; Newsham and Robinson, 2009). Whilst the synthesis of UV photoprotective compounds is less studied in bryophytes than vascular plants, it still appears to be one of the most common plant responses to elevated UV-B exposure (Searles et al., 2001; Searles et al., 2002; Newsham and Robinson, 2009). Accumulation of photoprotective compounds in response to elevated UV-B radiation occurs in many mosses including *P. schreberi* (Lappalainen et al., 2008), *Bryum argenteum* (Markham, 1990), *Polytrichastrum alpinum*, *Funaria hygrometrica* and three Sphagnum species (Huttunen et al., 2005) as well as the Antarctic species *Bryum pseudotriquetrum* (Dunn and Robinson, 2006), *Andreaea regularis* (Newsham, 2003) and *Sanionia uncinata* (Newsham et al., 2002). Liverworts that showed similar trends include *Jungermannia exsertifolia* subsp. *cordifolia* (Arroniz-Crespo et al., 2011) and *Cephaloziella varians* (*exiliflora*) (Snell et al., 2009).

However, the synthesis of UVAC did not increase with increasing UV-B light in all moss species studied e.g. *Polytrichum commune* (Barsig et al., 1998; Gehrke, 1999), *Schistidium antarctici* (Dunn and Robinson, 2006), *Hylocomium splendens* (Gehrke, 1999; Taipale and Huttunen, 2002), *S. uncinata* (temperate species; Lud et al., 2002), *Polytrichum juniperinum* (Lappalainen et al., 2009), and *Sphagnum balticum* and *Sphagnum papillosum* (Niemi et al., 2002). The lack of UV absorbing pigments detected in some or all of these species may reflect the methodology used, which commonly only extracts the intracellular UVAC (Section IIC; Semerdjieva et al., 2003; Clarke et al., 2008). It is also possible that some species maintain a high level of UVAC compounds constitutively rather than producing them only in response to elevated UV-B radiation. Few studies have actually quantified the metabolic cost

to bryophytes of UVAC production, a study of the liverwort *C. varians* in Antarctica suggests the cost maybe relatively low (<2%; Snell et al., 2009).

Whether photoprotective compounds are induced by elevated levels of UV or are constitutively produced (Bornman, 1998), their presence in bryophytes is usually effective in maintaining optimal photosynthetic efficiency measured by chlorophyll fluorescence (Fv/Fm, a measure of plant stress). This is demonstrated in the photoprotection exhibited in Antarctic mosses *B. argentum* and *Ceratodon purpureus* (Green et al., 2005) and in the temperate mosses *H. splendens* and *P. commune* (Arroniz-Crespo et al., 2011). Similarly multiple regression analysis of the response of two Antarctic bryophytes (*S. uncinata* and *C. varians*) suggests that UV-B screening pigments protect against UV-B induced lowering of Fv/Fm in these species (Newsham et al., 2002). In contrast low concentrations of UVAC were found in the endemic Antarctic moss species *S. antarctici* (Dunn and Robinson, 2006; Clarke and Robinson, 2008) resulting in a lack of protection to PSII that could be causing photoinhibition when this moss is exposed to high UV-B radiation levels (Adamson et al., 1988) and contributing to its susceptibility to the ozone hole increased, UV environment (Turnbull et al., 2009; Turnbull and Robinson, 2009). An UV-B specific decline in Fv/Fm (under PAR +UVA+UVB as compared to PAR and PAR +UVA treatments) was also observed in two aquatic bryophytes, the moss *Fontinalis antipretica* and the liverwort *J. exsertifolia*, for the duration of a 36 day experiment (Martinez-Abaigar et al., 2003) possibly demonstrating direct UV induced photoinhibition of PSII as described by Takahashi et al. (2010).

Some bryophytes exhibit naturally green and red forms that change in response to differing UV environments. Generally, the red forms are found in exposed and drier sites and the morphologically similar green form grows in naturally shaded and wetter sites. Red forms of bryophytes appear more resistant to the damaging effects of UV radiation (Post, 1990; Post and Vesk, 1992; Hooijmaijers and Gould, 2007). For example, the red form of the liverwort *Jamesoniella colorata* maintained greater Fv/Fm, photochemical quenching (qP) and non-photochemical quenching (NPQ) than its green counterpart when exposed to UV-B radiation (Hooijmaijers and Gould, 2007). The red pigment in this liverwort was found to be tightly associated with the cell wall but has not yet been identified. Similarly, red anthocyanic pigmentation is evident within the Antarctic liverwort *C. varians* (Post and Vesk, 1992; Newsham, 2010) and the cell walls of red *C. purpureus* (Post, 1990; Green et al., 2005) and

may contribute to the greater resistance to UV-induced effects of the red rather than the green forms of these species.

C Structure of UV absorbing compounds in bryophytes

The ability of flavonoids, hydroxycinnamic acids and other photoprotective compounds to absorb within the UV-B range (280–315 nm) is based on their aromatic structures. The majority of these compounds are phenolics containing at least one aromatic ring, usually in the form of benzene, which allows high absorption in the UV range (Cockell, 1998). This absorption range is completely dependent on the structure and does not include photosynthetically active radiation (Schnitzler et al., 1996; Cove et al., 1997). Simple phenolics have one absorption peak in the UV region and more complex phenolics, like flavonoids, have two or more (Meijkamp et al., 1999). Peaks of absorbance are not only determined by the aromatic rings but also by the nature and position of any substituents.

Flavonoids, which are commonly found in plants including many bryophytes, have a backbone consisting of 15 carbons that form aromatic rings connected by a three carbon bridge (Swain, 1976; Koes et al., 1994). Flavonoids are divided into four prominent groups consisting of flavones, flavonols, isoflavones and anthocyanins. Various derivatives of these, hydroxycinnamic acids and other UV absorbing compounds have been found in polar and temperate bryophytes (Table 1).

Complex phenolics like flavonoids are derived from a combination of the shikimate and phenylpropanoid pathways (Koes et al., 1994). The phenylpropanoid pathway begins with the conversion of phenylalanine to cinnamic acid by phenylalanine ammonia-lyase (PAL; Fig. 4) Further catalysis by two other enzymes in the pathway leads to the formation of *p*-coumaroyl coenzyme A (CoA). The general flavonoid biosynthesis pathway in plants begins with chalcone synthase (CHS), an enzyme which catalyses the reaction between *p*-coumaroyl CoA (from the phenylpropanoid pathway) and three units of malonyl CoA (a product of the shikimate pathway). Cyclization results in the formation of a chalcone (naringenin chalcone). This initiates the development of complex phenolic compounds including flavonoids and lignin (Boelen et al., 2006). Whilst there is limited information regarding biosynthesis of flavonoids and other UV absorbing compounds in bryophytes specifically, genome sequences confirm that a CHS multigene family exists in *Physcomitrella patens* and there are similarities

between the enzymatic properties of CHS from this moss species and that of higher plants (Jiang et al., 2006).

The accumulation of flavonoids and other photoprotective compounds is most likely activated due to PAL, CHS and other enzymes involved in their production being stimulated by UV-B (Rozema et al., 2002; Rizzini et al., 2011; United Nations Environment Programme, 2012). There is also evidence suggesting that the genes encoding these enzymes can be up-regulated by UV radiation (Cooper-Driver and Bhattacharya, 1998; Ballare et al., 2011) as has been demonstrated in the moss *P. patens* (Wolf et al., 2010).

Within vascular plant cells, flavonoids are located in the cytoplasm, plastid membranes, vacuoles, nuclei and cell walls (Swain, 1976; Schnitzler et al., 1996; Agati et al., 2007). The majority of studies of UVAC in bryophytes have focused on the methanol-extractable or intracellular compounds. These are the most accessible for extraction and subsequent isolation and characterization. However, recent studies showing the presence of cell wall photoprotective compounds within bryophytes potentially indicates a more effective protective barrier against UV-B radiation. The UV tolerant *C. purpureus* is one such bryophyte that localizes the majority of its UVAC within its cell walls (Fig. 2; Clarke and Robinson, 2008). Although reports of photoprotective compounds bound to the cell walls of bryophytes or other plant species is unusual (Semerdjieva et al., 2003; Clarke and Robinson, 2008) this may reflect the lack of studies that have used alkaline digestion to extract these wall bound pigments rather than the absence of UVAC in these locations. Cell wall UVAC would function as a first defense barrier to UV radiation in bryophytes and could prove to be a more effective UV screen than intracellular UV absorbing compounds (Turnbull et al., 2009; Turnbull and Robinson, 2009). Two intermediates in the phenylpropanoid pathway, ferulic and coumaric acids have been isolated from the cell walls of *Mnium hornum* (Davidson et al., 1989). These compounds are acetylated within the cell to form polymers that can then be bound within the cell wall.

III Dealing with excess light absorbed within the chloroplast

If excess or damaging light is not absorbed by screening compounds in the cell wall or intracellularly there are mechanisms within the chloroplast that can also protect against photodamage. Absorption of excess PAR radiation could lead to accumulation of ROS, which

in turn inhibits the repair of damaged PSII. Prevention of ROS accumulation occurs through both dissipation of the energy prior to ROS formation and scavenging of any ROS that are produced. Photoprotective mechanisms that can reduce the production of ROS include thermal dissipation of light energy (Nichol et al., 2012), as well as pathways that consume the excess light energy such as cyclic electron flow and photorespiration (reviewed in Takahashi and Badger, 2011). The discrepancy between relatively low carbon fixation rates and the often non-saturating electron transport rates (measured by chlorophyll fluorescence) suggest that alternative electron sinks are an important component of photoprotection in many bryophytes (Proctor and Smirnoff, 2011).

A Dissipating excess energy as heat, non photochemical quenching and the xanthophyll cycles

If excess light is absorbed by the light-harvesting complexes (LHC) of PSII it can be dissipated as harmless heat energy (thermal energy dissipation; qE or non photochemical quenching NPQ). Thermal energy dissipation is associated with the activity of one or more xanthophyll cycles (reviewed in Nichol et al., 2012). The first of these involves the light dependent conversion of violaxanthin (V) to zeaxanthin (Z) via antheraxanthin (A) (Demmig-Adams and W. W. Adams, 1992); whilst the second involves the direct interconversion of lutein to lutein epoxidase (Fig. 5; Bungard et al., 1999; García-Plazaola et al., 2007). These conversions are catalyzed by the enzyme violaxanthin de-epoxidase and, in addition to the involvement of lutein or zeaxanthin, qE also requires protonation of the PSII protein subunit PsbS (an ortholog of this protein is present in mosses Alboresi et al., 2008). A low pH in the chloroplast lumen also enhances both the interconversion to the photoprotective form (L or Z) and the potential for thermal dissipation, which enables subtle switching of qE activity to correspond with the need for photoprotection (Niyogi, 1999). Recent work with Arabidopsis mutants suggests that qE acts to prevent photoinhibition by suppressing the formation of ROS, which would otherwise impair the processes that repair damaged PSII (Takahashi and Badger, 2011).

Sequence analysis of the antenna protein multigene family in *P. patens*, has shown that some antenna polypeptides, such as Lhcb6, are present only in land plants, suggesting they play a role in adaptation to the sub-aerial environment and more particularly in the formation of NPQ (Alboresi et al., 2008). In addition to PsbS, *P. patens* produces isoforms of another

protein (LHCSR), which is involved in formation of NPQ in algae (Alboresia et al., 2010; Gerotto et al., 2011). The presence of these two NPQ related proteins in *P. patens* suggests that the PsbS-dependent NPQ of plants evolved before the LHCSR based mechanism typical of the algal ancestor was lost. LHCSR was subsequently lost, in vascular plants, presumably as the newly evolved PsbS-dependent mechanism ensured a sufficient level of photoprotection (Alboresia et al., 2010).

Acclimation of *P. patens* to either high light or low temperature is accompanied by the ability to produce a strong, fast NPQ response associated with overexpression of both PsbS and LHCSR proteins (Gerotto et al., 2011). Mutants depleted of PsbS and/or LHCSR confirm that the NPQ response is associated with presence of these proteins and show enhanced photosensitivity when exposed to either high light or low temperature. Different isoforms of LHCSR appear to be involved in acclimation to either high light (LHCSR1) or low temperatures (LHCSR2, Gerotto et al., 2011).

Whilst the VAZ xanthophyll cycle has been shown to be present in many bryophytes (Deltoro et al., 1998; Lovelock and Robinson, 2002; Newsham et al., 2002; Robinson et al., 2005; Arroniz-Crespo et al., 2011), until recently the L/Lx cycle has received less attention. Lutein epoxide was found in leaves of 62% of the species specifically examined for this carotenoid (García-Plazaola et al., 2007) and its prevalence in shade plants and co-occurrence with ∝-carotene (another pigment associated with shade leaves) suggests it should be present in most shade inhabiting bryophytes (Matsubara et al., 2009). In a survey of 14 species of bryophytes using thin layer chromatography (TLC), Czeczuga and coworkers (2006) found the gametophytes contained up to 25 carotenoids, with β-carotene, β-cryptoxanthin, lutein, and lutein epoxide found in all species examined. Quantification and studies into the involvement of the L/Lx xanthophyll cycle in bryophytes will require the adoption of modified methods of high performance liquid chromatography (HPLC; Förster et al., 2009) since the shorter HPLC runs normally used to analyze the VAZ xanthophyll cycle pigments, tend to cause co-elution of pigments and can mask the presence of Lx.

Strong NPQ, often associated with de-epoxidation of V to Z, is common in bryophytes, especially those from sun-adapted habitats (Marschall and Proctor, 2004), and under desiccating (Deltoro et al., 1998) or freezing conditions (Deltoro et al., 1999) suggesting that they can dissipate excess light energy effectively. The epoxidation of Z back to V can also be

slow leading to sustained high levels of Z and the potential for fast activation of NPQ dependent on the ΔpH (Lovelock et al., 1995; Deltoro et al., 1998). The constitutive presence of Z is likely to be particularly important in those bryophytes that go through repeated cycles of desiccation and rehydration or freezing and thawing. A good example of priming of the xanthophyll cycle in the protective form has been demonstrated in desiccation tolerant species from a range of plant forms including mosses and liverworts (Fernandez-Marin et al., 2011). Slow desiccation, of paired desiccation sensitive (*Lunularia cruciata* and *Palustriella* sp.) and desiccation tolerant (*Frullania dilatata* and *Syntrichia ruralis*) species produced de-epoxidation of the xanthophyll cycle pigments in darkness, accompanied by a reduction in Fv/Fm. After re-wetting in darkness, the pigments were converted back to V in parallel with the recovery of Fv/Fm in both mosses and the desiccation tolerant liverwort, with the desiccation tolerant bryophytes both showing full recovery of initial Fv/Fm. The stability of the β-carotene pool confirmed that Z was produced from V and not by *de novo* synthesis. This ability to produce Z in the dark during dehydration presumably offers potential protection when bryophytes face sudden rehydration in the light.

Several groups (Heber et al., 2006; Heber et al., 2007; Nabe et al., 2007) have proposed that during slow desiccation another thermal energy dissipation mechanism is activated which requires neither protonation nor Z but acts alongside Z-dependent energy dissipation, providing desiccation occurs in the light. They attribute this to the formation of quenching PSII reaction centers in desiccated poikilohydric autotrophs (Heber et al., 2006). Such quenching centers might explain similar findings in the Antarctic moss *S. antarctici* during freezing (Lovelock et al., 1995; Lovelock et al., 1995). The extent to which this is related to LHCSR proteins remains to be elucidated (Gerotto et al., 2011).

B Consuming excess energy in the chloroplasts: cyclic electron flow, photorespiration and the Mehler reaction.

Processes that consume energy in the chloroplast effectively prevent the formation of ROS. Cyclic electron flow around PSI enhances the development of ΔpH across the thylakoid membrane and has been shown to play a role photoprotection via at least two mechanisms (reviewed in Shikanai, 2007; Takahashi and Badger, 2011).

Photorespiration, the oxygenation of ribulose -1,5-bisphospate (RuBP) by ribulose -1,5-bisphospate carboylase-oygenase (Rubisco) maintains energy utilization and can thus have a photoprotective function when carboxylation is limited by low CO_2 concentration. This could be particularly important in bryophytes since diffusion of CO_2 into leaves maybe limited by relatively unventilated leaf surfaces (compared to higher plant leaves) (Marschall and Proctor, 2004). Studies with sun exposed *Schistidium apocarpum* indicate a very high capacity for oxygen photoreduction when CO_2 assimilation is limited but suggest this is not photorespiratory in nature but more likely the Mehler-peroxidase reaction (water-water cycle; Asada, 2006; Proctor and Smirnoff, 2011). Since the Mehler reaction causes photoreduction of oxygen to hydrogen peroxide (H_2O_2) in Photosystem I this reaction depends on an effective ROS scavenging system (Asada, 2006).

If all these photoprotective mechanisms fail or the Mehler reaction is occurring and ROS are produced within the chloroplasts, oxidative stress can still be avoided if the ROS are effectively scavenged. Multiple enzymes, including superoxide dismutase and ascorbate peroxidase (and peroxiredoxin) and antioxidant compounds (e.g. ascorbate, ∝-tocopherol and carotenoids such as zeaxanthin, lutein and β-carotene) act as scavenging systems. These ROS scavenging systems have been demonstrated in mosses as in other plants (Dhindsa, 1991; Seel et al., 1992).

IV Conclusions

Despite being commonly associated with low light environments bryophytes generally show an impressive suite of photoprotective mechanisms most but not all of which are also common to vascular plants. Areas that stand out as requiring further study include; an assessment of the role of UV radiation in causing specific damage to PSII, analysis of the role of cell wall UVAC in screening damaging UV-B radiation, clarification of the roles of the PsbS and LHCSR proteins in nonphotochemical quenching and an investigation of the role of the L/Lx cycle in photoprotection in bryophytes. Determination of sequences for additional bryophyte species, such as *C. purpureus*, combined with targeted physiological and biochemical studies should ensure improved understanding of the evolution of photoprotective strategies in the land plants.

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Figure 1

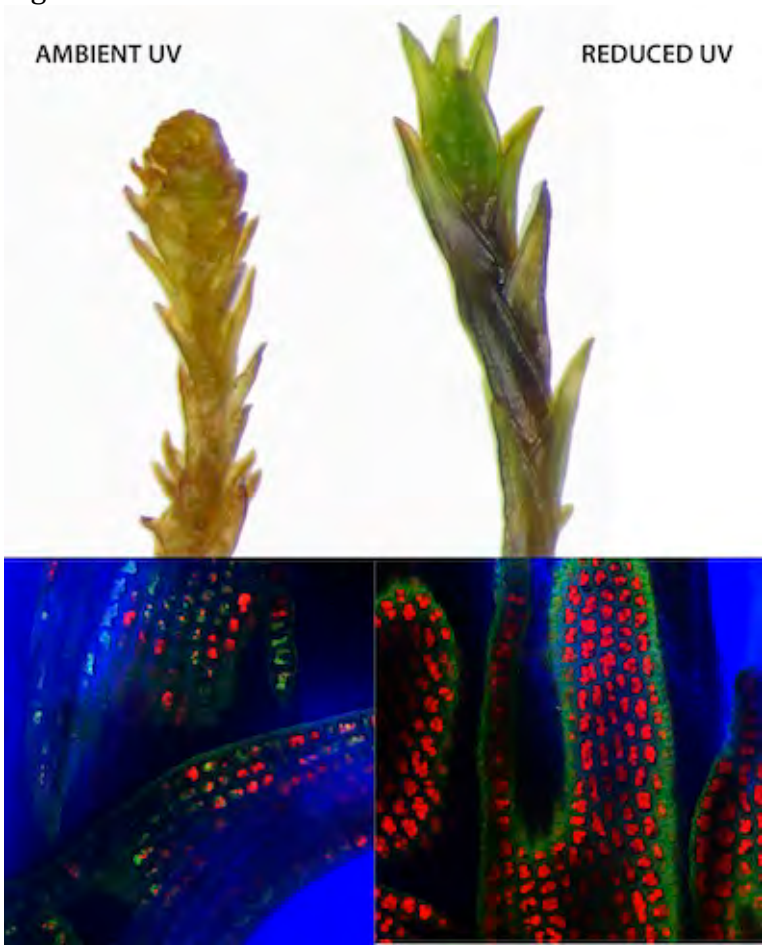


Figure 2

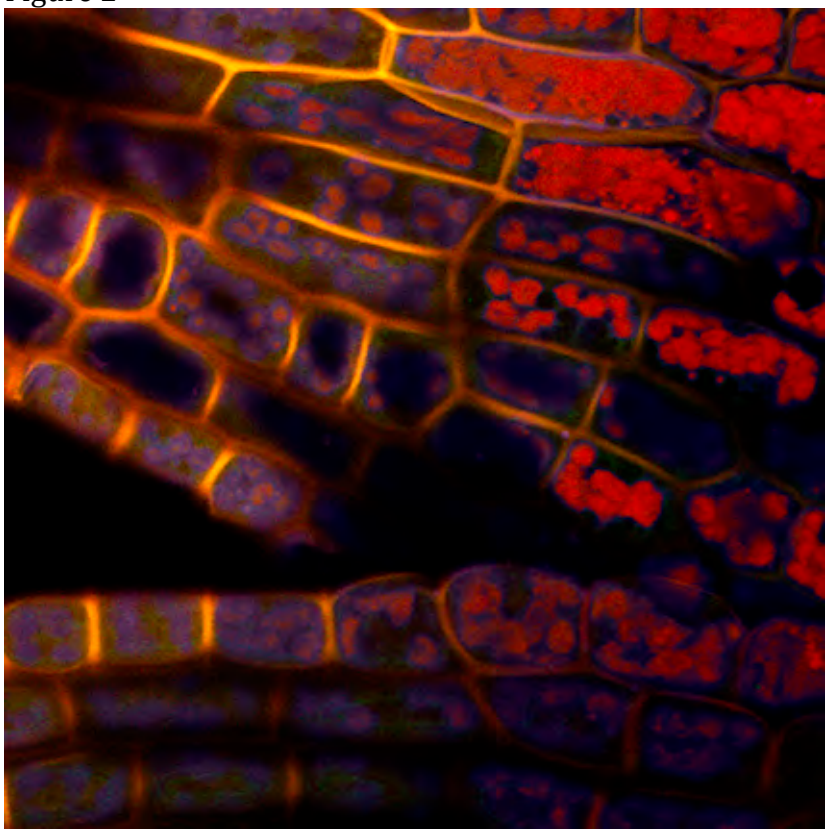


Figure 3

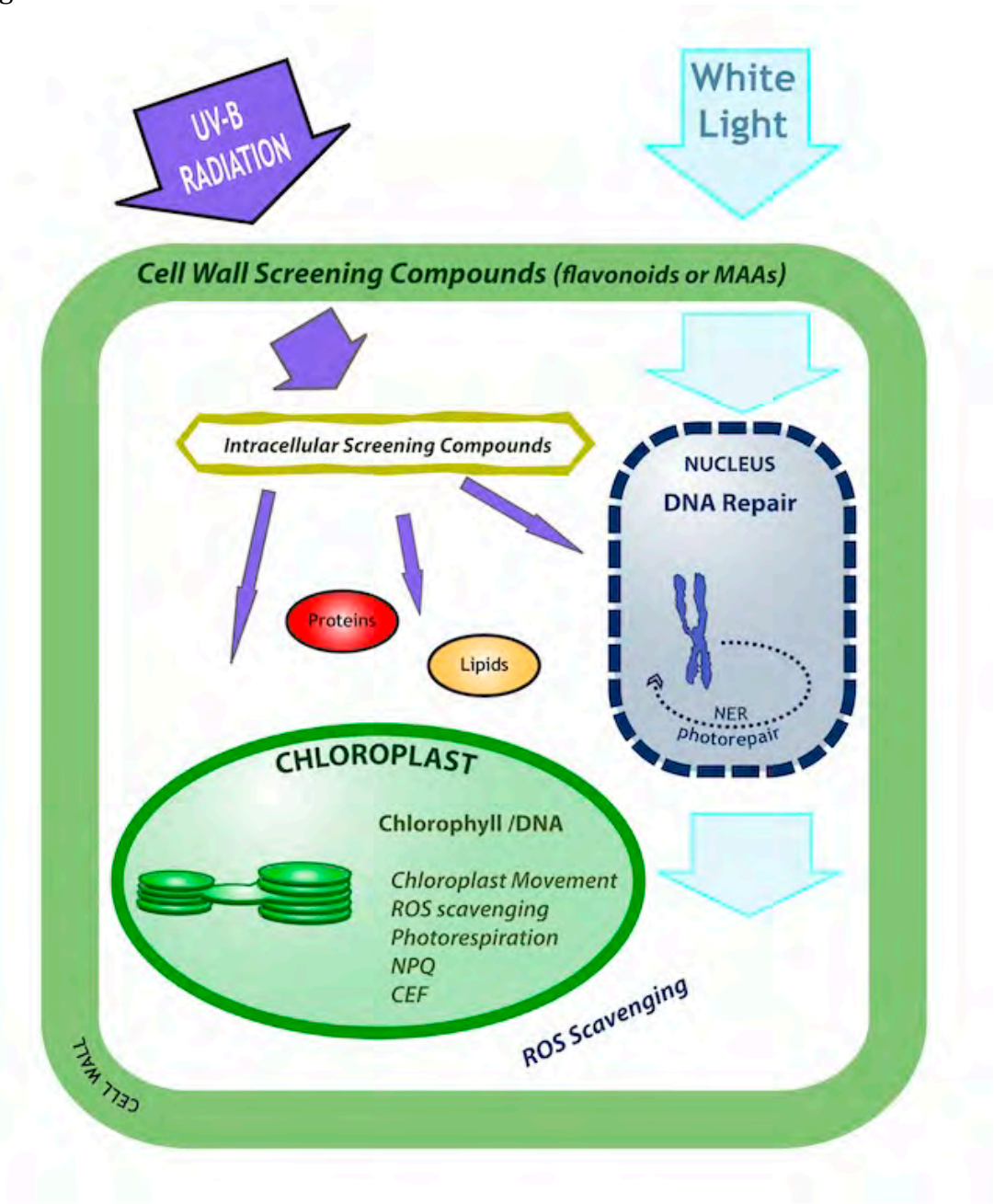

UV-B RADIATION
White Light
Cell Wall Screening Compounds (flavonoids or MAAs)
Intracellular Screening Compounds
NUCLEUS
DNA Repair
NER
photorepair
Proteins
Lipids
CHLOROPLAST
Chlorophyll /DNA
Chloroplast Movement
ROS scavenging
Photorespiration
NPQ
CEF
ROS Scavenging
CELL WALL

Figure 4

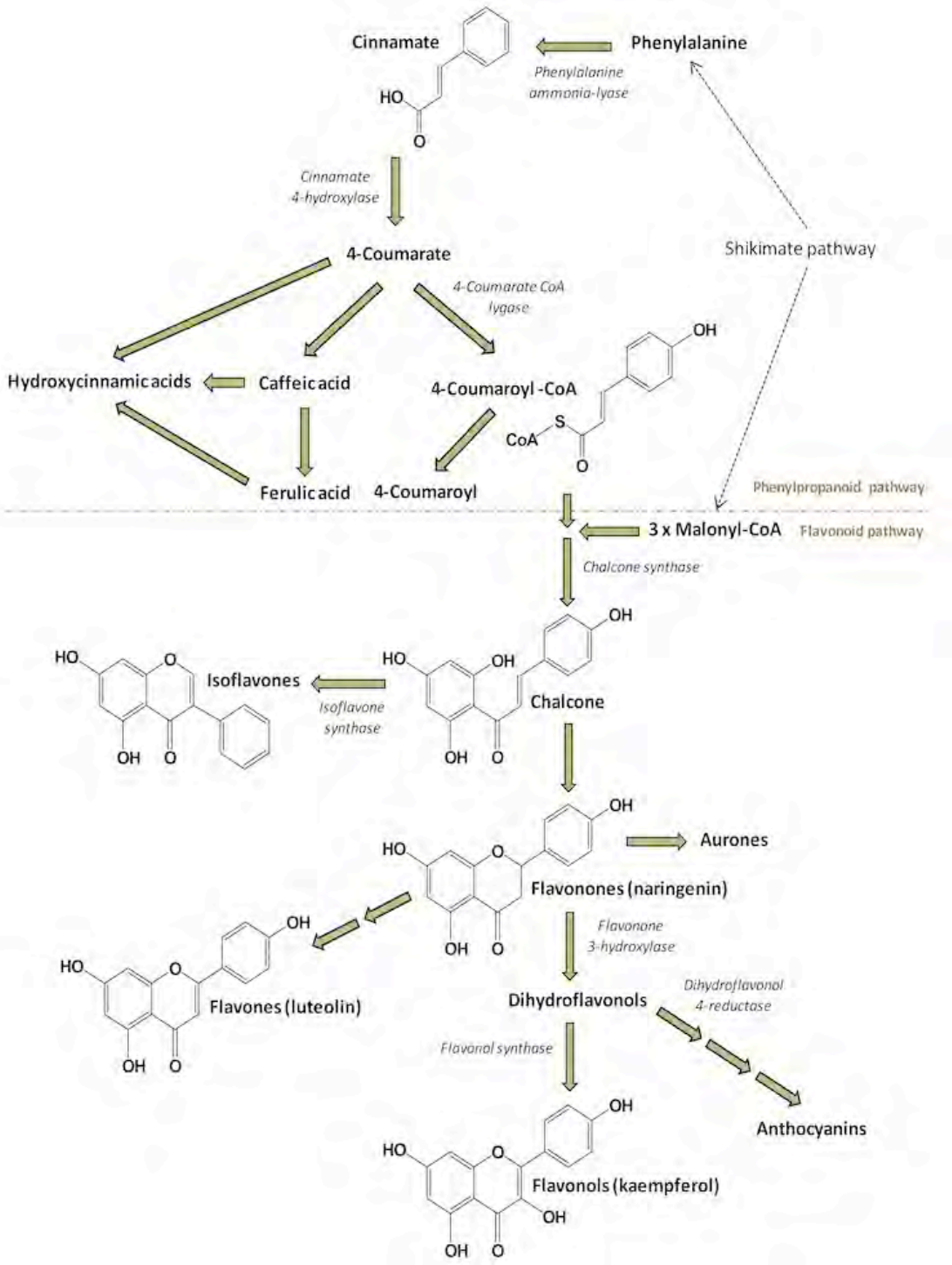
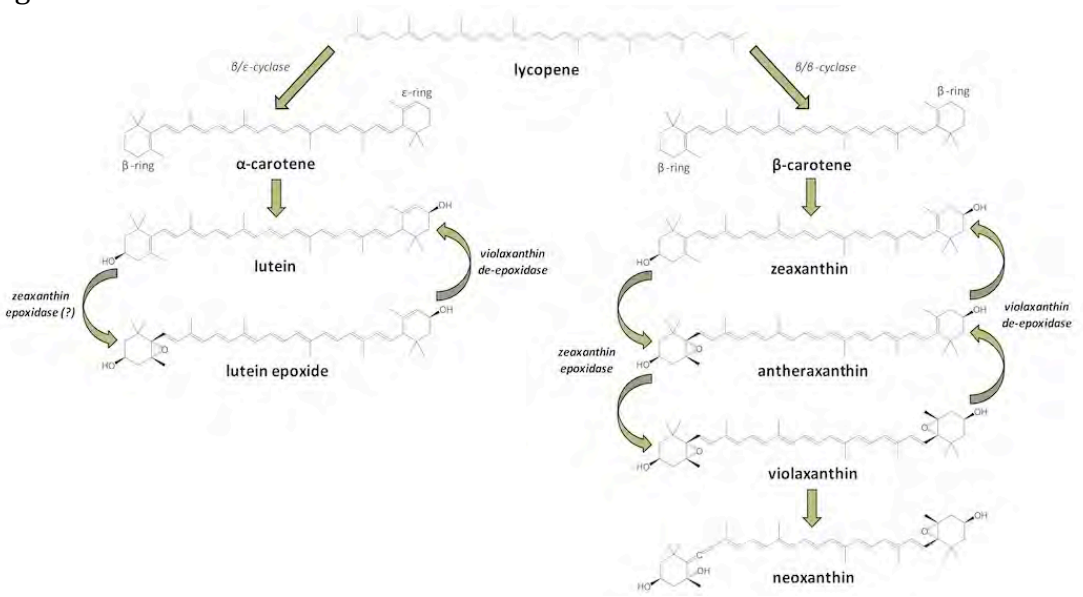

Cinnamate
Phenylalanine
Phenylalanine ammonia-lyase
Cinnamate 4-hydroxylase
4-Coumarate
Shikimate pathway
4-Coumarate CoA lygase
Hydroxycinnamic acids
Caffeic acid
4-Coumaroyl-CoA
CoA
Ferulic acid
4-Coumaroyl
Phenylpropanoid pathway
3 x Malonyl-CoA
Flavonoid pathway
Chalcone synthase
Isoflavones
Isoflavone synthase
Chalcone
Aurones
Flavonones (naringenin)
Flavonone 3-hydroxylase
Flavones (luteolin)
Dihydroflavonols
Dihydroflavonol 4-reductase
Flavonol synthase
Anthocyanins
Flavonols (kaempferol)

Figure 5


lycopene
β/ε-cyclase
ε-ring
β-ring
α-carotene
lutein
violaxanthin
de-epoxidase
zeaxanthin
epoxidase (?)
lutein epoxide
β/β-cyclase
β-ring
β-carotene
zeaxanthin
violaxanthin
de-epoxidase
zeaxanthin
epoxidase
antheraxanthin
violaxanthin
neoxanthin